PATENT SPECIFICATION

(11) 1 241 539

DRAWINGS ATTACHED

1 241 539

(21) Application No. 21283/70 (22) Filed 4 May 1970
(31) Convention Application No. 869 766 (32) Filed 27 Oct. 1969 in
(33) United States of America (US)
(45) Complete Specification published 4 Aug. 1971
(51) International Classification B 04 b 5/04 B 01 f 3/08 15/02
(52) Index at acceptance
B2P 10A 2 3A 9A3B 9E2
B1C 14 18E1 22 31 4 5 6 7

(54) CELL-WASHING CENTRIFUGE AND METHOD

(71) We, AMERICAN HOSPITAL SUPPLY CORPORATION a Corporation organized and existing under the Laws of the State of Illinois, United States of America, of 1740 Ridge Avenue, Evanston, Illinois, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The mixing and decanting centrifuge disclosed in United States patent 3,401,876 is provided with a rotary head which supports a multiplicity of test tubes containing blood cells. In the cell washing operation of that centrifuge (which is especially useful in connection with the Coombs blood test), the patent teaches that each of the tubes is engaged by a resilient finger and is rotated intermittently about its own axis as it travels about the central axis of centrifugation. Similarly, U.S. patent 3,235,173 teaches that for proper mixing of solid and liquid material, or of liquids, it is essential for the tubes to be rotated intermittently and at varying speed about their own axes to achieve proper mixing of their contents. Obviously, in the absence of complete mixing between blood cells and the washing fluid, the washing step would be ineffective and the results of any tests subsequently performed on such cells would tend to be adversely affected. Thus, the teaching that the tubes be rotated or agitated in some manner to assure thorough mixing of the material is consistent with standard manual practice in which each tube is manually shaken for a predetermined interval for the purpose of completely dispersing blood cells in the washing fluid (during preliminary washing steps) or in the Coombs serum or other testing fluid (during the final testing step).

The present invention relates to an automatic cell washer in which thorough mixing is achieved between cells and washing or treating fluids without rotating the test tubes about their own axes either intermittently or continuously, or at varying speeds or constant speed. In short, the step thought to be essential in prior automatic cell washing operations is completely eliminated. One aspect of the invention lies in the discovery that if washing or treating fluids is forcefully introduced into each tube at the open end thereof as such tube is travelling rapidly about an axis of centrifugation spaced therefrom, the fluid will accelerate down the outer wall of the tube and will disperse the cells in the tube's bottom outer portion. Thorough intermixing will occur because of the velocity of the fluid down the tube's outer wall portion and because the kinetic energy of the fluid will cause a displacement of the cells from their original positions. Furthermore, such mixing avoids cell damage which might result, and has in some instances been thought to occur and to cause non-specific agglutination, when tubes containing the cells and serum undergoing centrifugation are also rotated intermittently about their own axes.

An object of the present invention is therefore to provide an improved method and apparatus for washing and treating blood cells. In that connection, it is a specific object to provide an apparatus having a manifold assembly which not only assures uniform distribution of fluid to each of a plurality of tubes carried by the apparatus but which also discharges such fluid into the tubes with sufficient velocity to assure complete dispersion of cells without the tube agitating steps taught to be essential in the prior art.

Other objects and advantages of the invention will appear as the specification proceeds.

Figure 1 is a perspective view of a cell-washing centrifuge embodying the present invention;

Figure 2 is an enlarged fragmentary perspective view of the centrifuge's hood assembly with portions of the cover and manifold

broken away to illustrate details thereof;

Figure 3 is a vertical sectional view showing the essential elements of the apparatus and illustrating in schematic form some of the standard components;

Figure 4 is an enlarged horizontal sectional view taken along line 4—4 of Figure 3;

Figure 5 is a top plan view of the tube-supporting head;

Figure 6 is an enlarged fragmentary vertical sectional view illustrating the relationship of parts at the commencement of a washing or treating operation;

Figure 7 is a sectional view similar to Figure 6 but illustrating a subsequent step;

Figure 8 is similar to Figures 6 and 7 but illustrating a still further step;

Figure 9 is similar to Figures 6—8 but illustrates a final step in the operation of the unit.

Referring to the drawings, the numeral 10 generally designates a cell-washing centrifuge having a generally rectangular casing 11, a hood assembly 12, and a tube-supporting head assembly 13 (Figure 3). A reagent, normally a washing fluid such as an isotonic saline solution, is supplied to the apparatus from a suitable source 13. In Figure 1, the reagent supply source 13 is illustrated as a bottle suspended from a standard 14 which is mounted on casing 11. Tube 15 leads to a suitable valve 16 which controls the flow of reagent in the manner disclosed hereinafter, and then leads to hood assembly 12 for distribution of the reagent to each of the test tubes.

As shown in Figure 3, the top wall 17 of the casing is provided with a well 18 of generally cylindrical shape. The tube-supporting head 13 is also of cylindrical shape and is disposed within well 18. The head is axially bored for slidably receiving a hollow drive shaft 19 which projects upwardly through opening 20 in the well. A friction drive member 21 is fixed to the drive shaft 19 directly beneath head 13 and is provided with a slip-resistant top surface layer 22 of rubber or other suitable material which frictionally engages a similar layer 23 secured to the underside of head 13. Thus, driving force may be applied to the head 13 when the head is in the position illustrated in Figure 3 even though the head may be readily removed from the well, and from the shaft or spindle 19 which it slidably receives, when the hood assembly 12 has been raised and swung laterally into the broken-line position illustrated in Figure 1.

Shaft 19 is supported and driven by a suitable driving means 24 such as an electric motor. Upon energization of the driving means, head 13 is rotated at a selected speed within the range of approximately 1,000 to 3,000 revolutions per minute (rpm). The preferred range for the washing and treating of cells in connection with the Coombs test is 1500 to 2300 rpm, with highly effective results being obtained at a speed of approximately 1900 rpm.

From Figures 3 and 5 it will be seen that head 13 is provided with a circumferentially-spaced series of openings or recesses 25 which extend downwardly from the top surface of the head and which receive the lower portions of test tubes or sample tubes 26. Each recess has a substantially vertical outer surface 25a and an upwardly and inwardly sloping inner surface 25b, the slope of the inner surface being within the range of 25 to 45 degrees from the vertical. In the embodiment illustrated in the drawings, the angle of surface 25b is approximately 36 degrees. Thus, the recesses 25 are radially elongated at their upper ends to permit pivotal movement of the test tubes 26 between the inwardly inclined position illustrated in Figures 3 and 6—8, and the upstanding or substantially vertical position illustrated in solid lines in Figure 9. If desired, the lower ends of the recesses may also be radially elongated (but a lesser extent than the upper ends thereof) to permit inward displacement of the lower ends of the tubes as shown by the broken lines in Figure 9. While such inward displacement is not an essential feature of the apparatus with respect to its mixing and decanting functions, it permits the removable head to be utilized as a tube storage rack when the head is removed from the well, and to permit manual filling of the tubes when the head is either in or out of the well, while at the same time maintaining the tubes in stable upstanding positions.

To facilitate removability, the head is provided with an upstanding axial stem 27 having an enlarged upper end 27a. The stem may thus be used as a handle in removing the head from the well and in replacing it therein. It will be observed that the upper end of the stem has a bore 28 of smaller diameter than the lower axial bore of the head which slidably receives shaft 19, and that the stem is provided with a key 29 for locking the head against rotation relative to the vertical splined shaft 30 of hood assembly 12.

The hood assembly consists essentially of an arm 31 pivotally carried by upstanding shaft 32 which projects upwardly through the casing's top wall and which is connected at its lower end to a hood lifting and pivoting mechanism 33. Figures 3 and 6—8 illustrate the hood assembly in its fully lowered position, whereas Figure 9 illustrates the hood assembly in a partially raised or intermediate position. The broken lines in Figures 1 and 3 show the hood in its fully raised position and, in that position, the

hood is capable of being pivoted about the axis of shaft 32 into the laterally-displaced position illustrated in broken lines in Figure 1. The hood lifting and pivoting control mechanism 33 may be constructed to shift the hood into the operative position (with shaft 30 in alignment with drive shaft 19) upon the depressing of button 34 on front panel 35. Pivotal and axial movement of shaft 32 through operation of driving mechanism 33 is synchronized with the operation of head-driving motor 24 through suitable timing and synchronizing means 36 schematically illustrated in Figure 3. The synchronizing means also synchronizes and controls operation of the reagent flow control 16 which may, if desired, take the form of a solenoid-operated flow control valve. Since means 16, 24, 33 and 36 are disclosed in greater detail in co-owned U.S. patent 3,401,876, and since such means does not constitute the invention herein, a more detailed disclosure thereof is believed unnecessary.

Referring to Figure 3, the enlarged free end portion 31a of hood arm 31 is equipped with bearing elements 37 and 38 for rotatably supporting the splined vertical shaft 30. Fixed to the intermediate portion of shaft 30 directly beneath arm 31 are distributor 39 and cover 40, the latter elements being illustrated most clearly in Figures 2 and 3.

Distributor 39 is generally cylindrical in shape, having a cylindrical side wall 41, a bottom wall 42, and a top wall 43. The distributor also has an upstanding axial sleeve portion 44 which is formed integrally with bottom wall 42 and which is fixed to an upstanding internal stem 45 of the dome-shaped cover 40. The sleeve 44, along with side, bottom and top walls 41—43, define an annular manifold chamber 46. Top wall 43 has a central opening 47 of substantially greater diameter than stem 44, thereby defining an annular inlet for the distributor. Reagent tube 15 extends through the free end portion of arm 31 and projects into annular opening 47 for the purpose of supplying fluid to the distributor.

A plurality of uniformly and circumferentially-spaced nozzles 48 project downwardly from the bottom wall 42 of the distributor, projecting through openings 49 in cover 40. A generally vertical passage 50 extends through each nozzle and upwardly through side wall 41, as illustrated clearly in Figures 2 and 6—8. Figure 4, which illustrates the distributor with top wall 43 removed, reveals that each vertical passage 50 is located in an enlarged portion 51 of the side wall, the enlargement appearing as a bulge along the inner surface of side wall 41, each bulge being spaced by a recessed surface portion 52. Generally horizontal inlet portions 50a of passages 50 extend along the upper end of the side wall in the direction of rotation of the distributor as represented by arrow 53. Inlet passage portions 50a communicate with the manifold chamber through openings in the walls of recesses 52.

In addition to its enlargements 51 and recesses 52, the inner surface of the distributor's side wall 41 slopes upwardly and outwardly at an angle within the range of 2 to 10 degrees from the vertical. An angle within the range of 4 to 6 degrees is preferred, although variations would occur depending upon the intended rotational speed of the unit, the fluid to be distributed, etc. For distributing isotonic saline as the washing fluid in the unit illustrated in the drawings, an angle of approximately 5 degrees has been found particularly effective.

Cover 40 is affixed to the lower end of manifold 39 and is provided with an internal annular shoulder 54 which engages the upper ends of test tubes 26 when the tubes are in the inclined positions of Figures 6—8 and the unit is performing its cell-washing cycle of operation. It will be observed that the open upper ends of the test tubes are positioned directly beneath nozzles 48 to permit automatic filling of the tubes when they are in their inclined positions. The cover also has an outwardly and downwardly inclined undersurface which is slidably engageable with the upper ends of the test tubes to permit gradual outward pivoting movement of such tubes under the influence of centrifugal force as the cover (as part of arm assembly 12) is slowly raised during rotation of head 13. Automatic decanting of supernatant therefore occurs, all as described in detail in U.S. patent 3,401,876 mentioned above.

Figures 6—9 illustrate in somewhat schematic form the essential steps of the cell-washing procedure. Test tubes 26, each containing a quantity of blood cells 60 (i.e., blood which includes the cellular components), are placed in the recesses of head 13 with the tubes in the inclined positions illustrated in Figure 6. The head is properly positioned within well 18 and starting button 34 is depressed to lower the hood assembly 12 into the operating position shown. Washing fluid, which may be isotonic saline, flows into manifold chamber 46 through tube 15, filling the chamber to approximately the level illustrated in Figure 6. Thereafter, the main motor 24 for driving the head 13 commences its operation. Since the manifold, cover, and head are all interlocked for simultaneous rotation by splined shaft 30, the washing fluid 61 in the manifold is forced outwardly under the influence of centrifugal force and into passages 50 through the intercommunicating inlet ports 50a (Figure 7). The fluid is thus discharged from depending nozzles 48 into

the inclined test tubes 26 disposed directly therebelow.

It has been found that distributor 39, with its recessed inside wall surface, is highly effective in achieving uniform distribution of washing fluid to the multiple tubes 26. Internal enlargements 51 are believed to contribute significantly in achieving a rapid acceleration of the fluid within the manifold as rotation of the distributor commences, thereby insuring that each recess receives substantially equal volumes of fluid during distribution. In addition, the upward and outward slope of the distributor's side wall surface plays an important part in the rapid and complete emptying of the manifold chamber and in imparting to the washing fluid sufficient velocity to insure complete mixing of the fluid and the cells to be washed.

As the head 13 commences rotation, centrifugal force drives cells 60 outwardly as indicated by arrow 62 in Figure 7. At the same time, fluid 61 from the distributor accelerates down the outer portion of the tube's inside surface in the direction represented by arrow 63. The result is a complete dispersing of the cells 62 in the washing fluid 61 (Figure 8). Continued operation of the centrifuge thereafter causes a settling and compaction of the washed cells in the bottom of the tube, at which time the hood assembly is lifted to permit outward pivoting of the tubes and an automatic decanting of the fluid (Figure 9). The washed cellular components remain in the bottom of the tube and, as in the Coombs test, may again be washed by automatic repetition of the above-described steps.

Following the decanting step illustrated in Figure 9, the cells at the bottom of the tube are in tightly compacted condition. Neverthe less, during a repetition of the washing steps of Figures 6—8, it has been found that such cells are completely re-suspended by the washing fluid injected into the tubes from the nozzles of the distributor. A highly effective washing operation occurs without rotation of tubes 26 about their own axes. Such tubes, restrained against rotation about their own axes by reason of frictional contact with the cover 40 and head 13, travel only about the central axis of centrifugation —that is, the axis of drive shaft 19.

Fluid discharged from the tubes during the decanting step illustrated in Figure 9 flows downwardly along the inner surface of cover 40 and into well 18, where it is carried away by drain tube 64 to a suitable waste receiver 65 (Figure 3). After the washing procedure has been completed (in the Coombs test, three such washing steps take place), the cells are exposed to whatever treatment fluid is required for the particular test involved. Thus, in performing a Coombs test utilizing the apparatus of this invention, an operator simply introduces measured amounts of Coombs serum into each of the tubes containing washed cells and the tubes are then centrifuged for a predetermined interval without saline injection and without the final decanting step of Figure 9.

Figure 6 illustrates the step of filling manifold chamber 46 while head 13 and distributor 39 are stationary; however, it is to be understood that effective results are obtained if fluid is supplied to the distributor while it is being rotated. Specifically, it has been found that substantially uniform distribution of washing fluid to each of the test tubes 26 occurs even if fluid is supplied through line 15 while the centrifuge is operating as shown in Figure 7. Particularly effective results are obtained if washing fluid is supplied in two stages, the first stage occurring as shown in Figure 6 while the distributor is stationary and the second stage occurring when the distributor is being rotated and the initial supply has been distributed to the sample tubes. Under such conditions, the second stage injection of fluid washes from the inside wall surfaces of the test tubes any blood cells which may have dried thereon during centrifugation.

While in the foregoing we have disclosed an embodiment of the invention in considerable detail for purposes of illustration, it will be understood by those skilled in the art that many of these details may be varied without departing from the scope of the invention as defined in the appended claims.

WHAT WE CLAIM IS:—

1. A method of mixing blood cells in the bottom of an elongated open-topped centrifuge tube with a suspending liquid, comprising the steps of introducing said liquid into the open top of an inclined centrifuge tube while the tube is travelling about a vertical axis of centrifugation spaced therefrom in the direction of the tube's open top, and simultaneously restraining movement of said tube about its own axis, whereby, said liquid accelerates under the influence of centrifugal force along the inside surface of said tube from the open top thereof and into forceful contact with said cells at the tube's bottom to dislodge said cells and mix thoroughly therewith.

2. The method of Claim 1 wherein there are the further steps of discontinuing the flow of said liquid, and thereafter continuing movement of said tube about said axis of centrifugation until said cells are compacted in the bottom of said tube.

3. The method of Claim 2 in which there is the further step of decanting liquid from said tube above said compacted cells and

thereafter repeating all of said steps until said cells are thoroughly washed.

4. A method of washing blood cells supported in the bottom of an elongated open-topped centrifuge tube with a washing liquid, comprising the steps of directing a stream of washing liquid against the upper portion of the inner surface of said centrifuge tube while said tube is inclined in the direction of an axis of centrifugation and is travelling about said axis, and simultaneously restraining movement of said tube about its own axis, whereby, said liquid accelerates under the influence of centrifugal force along said inner surface from the upper portion of said tube to the lower portion thereof and into forceful contact with said cells to dislodge said cells and mix thoroughly therewith.

5. The method of Claim 4 wherein there are the further steps of interrupting the flow of said washing liquid, and continuing movement of said tube about said axis of centrifugation until said cells are compacted in the bottom of said tube.

6. The method of Claim 5 in which there is the further step of decanting liquid from said tube above said compacted cells and thereafter repeating all of said steps until said cells are thoroughly washed.

7. A centrifuge for use in the method defined in any of Claims 1 to 6, having a rotatable head supporting a plurality of upstanding open-topped centrifuge tubes arranged in a circumferential series about, and inclined relative to the axis of, said head, a distributor disposed above said head and rotatable therewith, said distributor having a plurality of nozzles projecting downwardly into said inclined tubes for distributing suspending liquid thereto, and having a top wall, a bottom wall, and a generally cylindrical side wall having a circumferential series of vertical passages communicating with said nozzles at their lower ends and communicating with the interior of said distributor adjacent to the inner surface of said top wall; and said side wall having a generally upwardly and outwardly sloping inner surface; and means for restraining rotational movement of said inclined tubes about their own axes during rotation of said head.

8. The centrifuge of Claim 7 in which said passages are uniformly and circumferentially spaced within said side wall, the side wall's inner surface being provided with recesses of uniform size and shape disposed between the passage-providing portions of said side wall and extending from said bottom wall to said top wall, each passage communicating at its upper end with a manifold chamber through a generally horizontal inlet portion of said passage opening into one of said recesses in said top wall.

9. The centrifuge of Claim 8 in which each of said inlet portions extends from the passage thereof in the direction of rotation of said distributor.

10. The centrifuge of Claim 7 in which said top wall is provided with an opening adjacent the axis of said distributor, and means for discharging measured amounts of suspending liquid into said distributor through said opening.

11. A cell-washing centrifuge for use in the method defined in any of Claims 1 to 6, having a rotatable head supporting a plurality of upstanding open-topped centrifuge tubes arranged in a circumferential series about, and inclined relative to, the axis of said head, said inclined tubes each being pivotal between an inwardly and upwardly sloping position and a substantially vertical position, and a cover engaging the upper ends of said inclined tubes and rotatable with said head, said cover mounted for gradual vertical movement from a lowered position restraining said inclined tubes in said inwardly and upwardly sloping positions and a raised position, wherein said inclined tubes are permitted to pivot gradually outwardly into said substantially vertical positions to cause the contents thereof to settle under the influence of centrifugal force; wherein a distributor is mounted upon said cover for supplying uniform amounts of washing liquid to said inclined tubes; said distributor having a chamber defined by a generally cylindrical side wall, a bottom wall, and a top wall; a plurality of tube-filling nozzles projecting downwardly from said bottom wall adjacent to the periphery thereof for supplying liquid to said inclined tubes; and passages extending through said nozzles and through said cylindrical side wall, each of said passages communicating with a manifold chamber only at the upper end thereof adjacent to said top wall; whereby liquid in the lower portion of said distributor chamber is distributed to said inclined tubes only when said distributor is rotated at a speed sufficient to cause said liquid to flow outwardly and upwardly under the influence of centrifugal force, and means for restraining rotational movement of said inclined tubes about their own axes during rotation of said head.

12. The centrifuge of Claim 11 in which said top wall is provided with an opening adjacent to the axis of said distributor; and means for discharging measured amounts of liquid into said manifold through said opening.

13. The centrifuge of Claim 11 in which said side wall of said distributor has an

inner surface which slopes upwardly and outwardly.

14. The centrifuge of Claim 13 in which said slope of said inner surfaces falls within the range of 2 to 10 degrees measured from the vertical.

15. The centrifuge of Claim 11 in which said passages are uniformly and circumferentially spaced within said side wall; the side wall's inner surface being provided with recesses of uniform size and shape disposed between the passage-providing portions of said side wall and extending from said bottom wall to said top wall; each passage communicating at its upper end with said chamber through a generally horizontal inlet portion opening into one of said recesses in said top wall.

16. The centrifuge of Claim 15 in which said side wall of said distributor has an inner surface which slopes upwardly and outwardly.

17. The centrifuge of Claim 16 in which said slope of said side wall is within the range of 2 to 10 degrees measured from the vertical.

18. The centrifuge of Claim 15 in which each of said inlet portions extends from said passage therefor in the direction of rotation of said distributor.

19. A method of mixing blood cells with a liquid substantially as hereinbefore described with reference to the accompanying drawings.

20. A centrifuge substantially as hereinbefore described with reference to the accompanying drawings.

21. A method of washing blood cells substantially as hereinbefore described.

CLEVELAND AND JOHNSON,
Chartered Patent Agents,
Agents for the Applicants,
Chancery House,
Chancery Lane,
London, WC2A 1QU.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon), Ltd.—1971.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY
from which copies may be obtained.

1241539 COMPLETE SPECIFICATION

4 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

FIG. 1

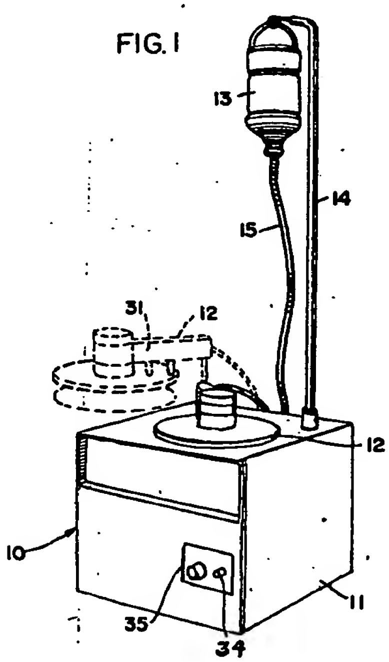


FIG. 6

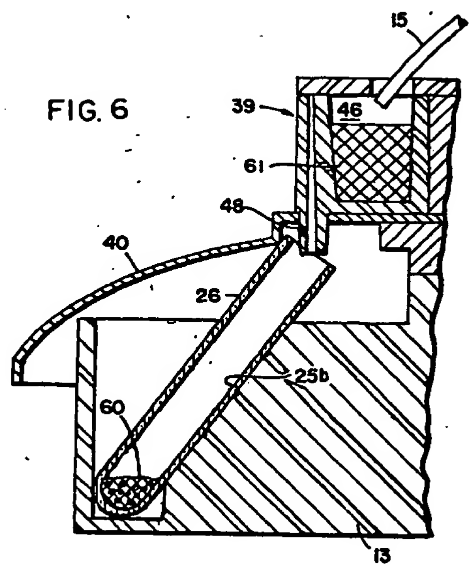


FIG. 4

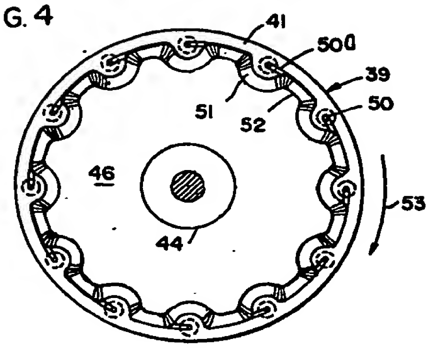
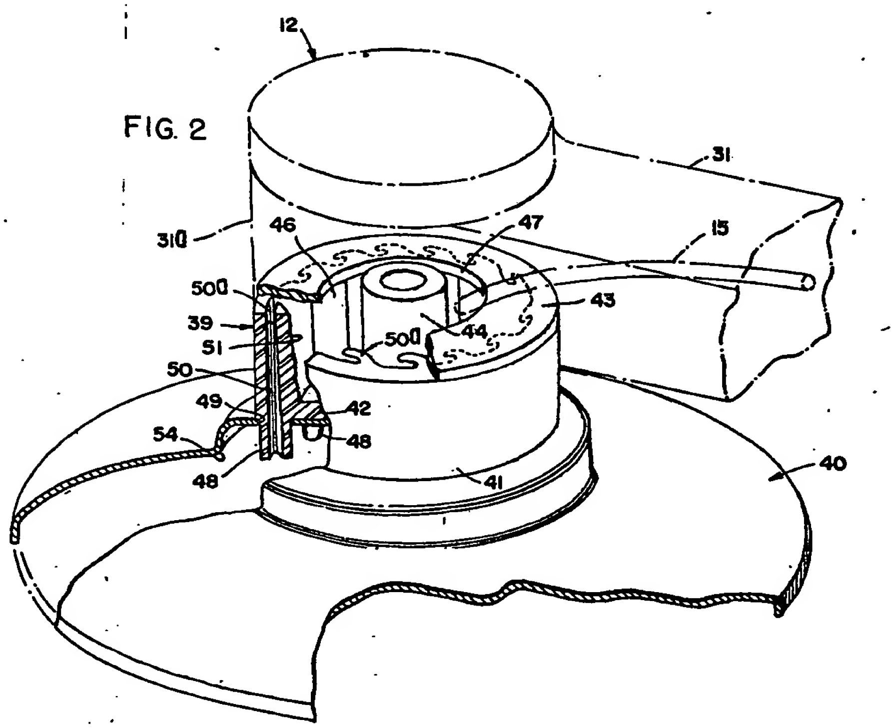

41
50a
39
50
51
52
46
53
44

FIG. 2


12
31
31a
46
47
15
50a
43
39
51
50a
44
50
49
42
54
48
48
41
40

1241539 COMPLETE SPECIFICATION

4 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 3

FIG. 3

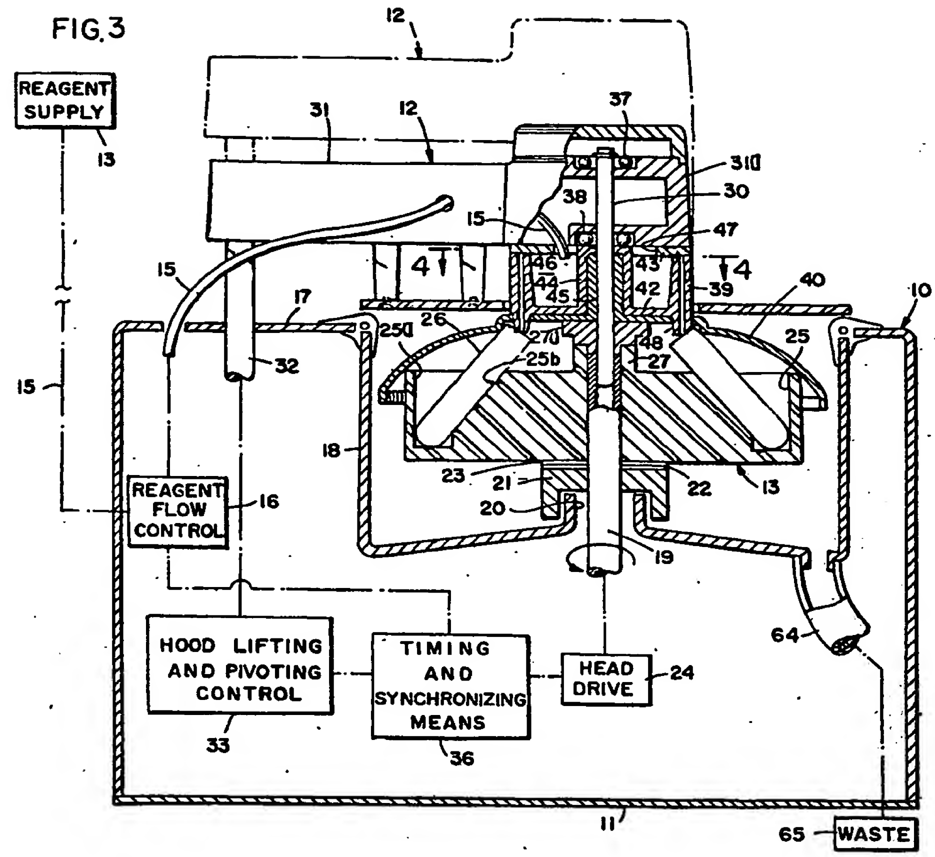
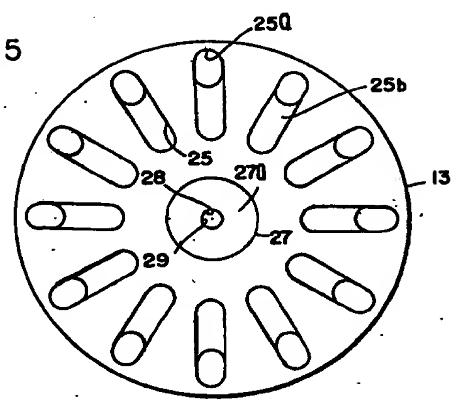


FIG. 5



1241539 COMPLETE SPECIFICATION
4 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 4

FIG. 7

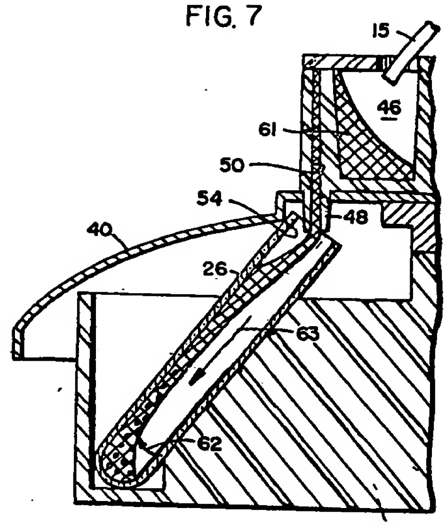


FIG. 9

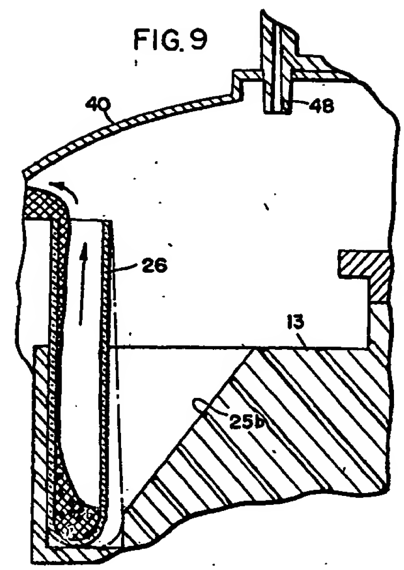


FIG. 8